The Bioscan 7(2) : 251-254, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

IN VITRO PROPAGATION OF *JATROPHA CURCAS* FROM EMBRYO AND NODAL EXPLANTS

G. D. RAM[1]*, S. S. CHAUHAN AND D. K. VERMA
[1]Department of Tissue Engineering, JSBB, SHIATS, Allahabad - 211 007 (UP)
Department of Molecular and Cellular Engineering, JSBB, SHIATS, Allahabad - 211 007 (UP)
E-mail: gd_bt@rediffmail.com

KEY WORDS
Jatropha curcas
In vitro
Nodal explants
Regeneration

Received on :
18.02.12

Accepted on :
28.04.12

***Corresponding author**

ABSTRACT

In vitro propagation of *Jatropha curcas* was established from embryo and node cultures of the field grown plants on Murashige and Skoog's medium supplemented with different composition of growth regulators *i.e.* Benzylaminopurine (BAP), Naphthalene acetic acid (NAA), and Indole-3-butyric acid (IBA). The modified MS medium BAP (3.0 mg/L) and IBA (1.0 mg/L) induced regeneration of maximum number of shoots (5-6 $\pm$ 1.48), whereas the maximum callus and shoot buds (5-6 $\pm$ 2.1) were produced on BAP (1.5 mg/L) and IBA (1.0 mg/L). The rooting and plantlet establishment were observed at IBA (3.0 mg/L) with maximum survival rate (95%).

INTRODUCTION

Jatropha curcas L. (Common name- Ratanjyot) is a multipurpose, stress resistant, zero-waste perennial and monoecious plant. It is one of the important plants of family Euphorbiaceae which is considered as a potential source of non-edible fuel-producing plant.

In recent years, this plant has received extensive attention due to medicinal significance and for its seed oil as commercial source of fuel (Datta and Pandey, 1993). The superior quality oil can be extracted from the seeds. The oil can be used as a mixed fuel for diesel/gasoline engines (Yoshifumi, 1982). The oil is not edible due to the presence of toxic substance "Curcascine". It is conventionally used in making soaps, candles, paints, lubricants and medicinally as a purgative (Dastur, 1952; Sujatha and Mukta, 1996).

Conventionally, the seeds and cuttings are commonly used for its propagation but the heterozygosity in seeds and the seasonal limitation of cuttings are the major constraints. It has also been reported that vegetative cuttings are not deep-rooted and are easily uprooted as they do not form a taproot system (Sujatha *et al.*, 2006).

To overcome the above mentioned difficulties, *in vitro* cultivation of the *Jatropha* sp. is the better option as it offers rapid and continuous supply of planting material and the evaluation of micropropagated *J. curcas* has revealed that they were at par with seed propagated plants in terms of yield and yield related traits (Sujatha and Mukta, 1996)

In vitro culture studies were undertaken in different species of *Jatropha*. Morphogenesis from endosperm tissues has been reported in *J. panduraefolia* (Srivastava, 1971; Johri, and Srivastava, 1973; Srivastava and Johri, 1974) High frequency regeneration from various explants of *J. integerrima* has been reported (Sujatha and Dhingra, 1993). Using different explants, plant regeneration protocols have also been described in *Jatropha curcas* (Sujatha and Mukta, 1996; Qin *et al.*, 2004; Rajore and Batra, 2005; Sujatha *et al.*, 2006), but multiplication rate was low for field applications.

In the present investigation, our objective was to improve the micropropagation efficiency using embryo and nodal explants. It has been reported that nodal meristems are an important source tissue of micropropagation and plants raised from these are comparatively more resistant to genetic variation (Pierik, 1991) and the disease free plants can be regenerated.

MATERIALS AND METHODS

All the experiments conducted in the present investigation were performed in the Department of Tissue Engineering, Jacob School of Biotechnology and Bioengineering, SHIATS, Allahabad and following materials and methodologies were employed:

Explant sources

Seeds of *Jatropha curcas* were collected from the Nursery, Department of Forestry, SHIATS, Allahabad, (UP). The seeds were treated with 0.1% Bavastin for 10 min. and washed with distilled water for five times. Seeds were again sterilized with 70% ethanol and 0.1% $HgCl_2$ for 45 sec. and 3 min respectively and rinsed again with sterile distilled water five times. The embryos extracted with scalpels in the Laminar Air

Flow chamber and inoculated on growth regulator-free MS medium with radicles in contact with medium, then epicotyls were taken from one-month-old *in vitro* grown plantlets and inoculated in MS with different concentrations and combinations of phytohormones.

Culture conditions

All explants were cultured on MS medium (Murashige and Skoog, 1962) with sucrose (3% w/v) and solidified with (0.8% w/v) agar. The pH was adjusted to 5.8±0.2 using 1N NaOH or 1N HCl solution before autoclaving. The cultures were incubated at 25°C±2°C in darkness or under 14h photoperiod using cool, white fluorescent lights (during subculture). Calli and regenerated shoots were recorded within 30 days.

Root induction

Single elongated shoots (1-2 cm long) were used for the induction of roots on MS medium under same conditions as for shoot induction. Root formation was evaluated after 30 days.

Transfer to soil

The regenerated plants with well developed shoots and roots were transferred to pots containing an artificial soil mixture for hardening at 25°C±2°C under diffuse-light (16/8-h photoperiod) conditions. To keep high humidity, potted plantlets were covered with polyethylene membranes and watered with liquid ½ MS medium free of sucrose every day. Two weeks later, the membranes were removed. The plantlets were acclimatized for another week and then transferred to a greenhouse.

Statistical analysis

Analysis of variance for all treatments in Randomized Block Design was carried out. The significance and non-significance of the treatment effect was judged with the help of 'F' variance ratio test. Calculated 'F' value was compared with the table value of 'F' at 5% level of significance. The significant differences between the means were tested against the critical differences at 5% level of significance. For testing the hypothesis, the ANOVA table was used.

RESULTS

Effect of various growth regulators on shoot regeneration

Explants cultured on MS medium containing growth regulators (BA/IBA/NAA) singly as well as in combination varied in response with respect to shoot length and number of shoot buds obtained per explant (Table 1). Explants remained green fresh but very insignificant shoot growth was observed *i.e.* 1.4 cm. in 3-4 weeks in growth regulator free medium. Maximum growth was found in treatment having composition MS + BAP (3.0 mg/L) + IBA (1.0 mg/L) which showed 5-6 shoot buds and 3.7 fold increase in shoot length over control (Fig. 1). This clearly indicated that BAP in combination with low concentration of auxin produced more number of shoots.

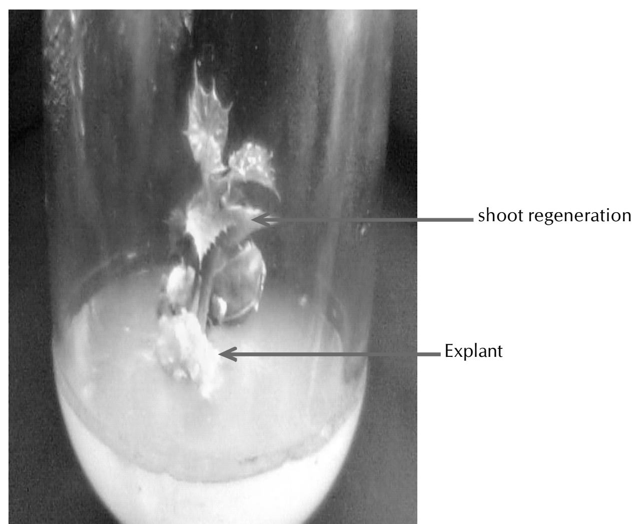

Figure 1: Shoot regeneration in MS medium supplemented with BAP (3.0 mg/L) and IBA (1.0 mg/L)

The effect of BAP and NAA in combination was also studied. MS with BAP (2.0 mg/L) + NAA (0.5 mg/L) produced 5-6 shoots showing 3.3 fold increases in shoot length over control. But by increasing the concentration callusing occurred which suppressed the growth of plant. When BAP was used alone in the MS medium, 2-3 shoots were obtained with 0.7 fold increase in shoot length over control.

Effect of various growth regulators on callus differentiation

Callus formation was observed within three to four weeks on embryo explant cultured on MS medium supplemented with varying concentration of plant growth regulators. Callusing was initiated after 7-10 days of inoculation. The callus induction always preceded by swelling of the explant. The phytohormones NAA, IBA and BAP were supplemented separately at different concentration to the MS basal medium (Table 2). The combination of BAP+IBA was found to be the best for producing green, compact and fast growing callus. Callus produced by IBA was green and later turned brown and showed limited growth. NAA (0.5-1.0 mg/L) was further tried with various concentrations of BAP and IBA to obtain well responses. It was observed that a combination of BAP

Table 1: Shoot regeneration on different hormonal combination.

Types of media	Hormonal combination (mg/L)			Time taken in weeks	No. of shoots	Shoot length (in cm.)
	BAP	NAA	IBA			
Control (M_1)	-	-	-	3-4	-	1.4
M_2	1.0	-	-	3-4	2-3	2.1
M_3	2.0	-	0.5	2-3	4-5	4.2
M_4	1.0	0.25	-	3-4	3-4	3.8
M_5	2.0	0.5	-	2-3	4-5	4.7
M_6	3.0	-	1.0	1.5-3	5-6	5.1

S. Ed. (±) 1.482; CD at 5% 2.996

Table 2: Effect of various growth regulators on callus differentiation

Types of media	Hormonal combination (mg/L) BAP	NAA	IBA	Callus induction	Time taken (weeks)	No. of shoot buds	Shoots length (cm.)
Control(M_1)	-	-	-	-	4-5	-	1.4
M_2	0.5	0.5	-	+	3-4	1-2	1.7
M_3	1.0	-	0.5	+ +	2-3	2-3	2.3
M_4	1.5	-	1.0	+ + + + +	2-3	5-6	4.7
M_5	1.5	-	1.5	+ + +	3-4	3-4	3.6
M_6	2.5	1.0	2.5	+ +	4-5	2-3	2.3

S. Ed. (±) 2.100; CD at 5% 4.245

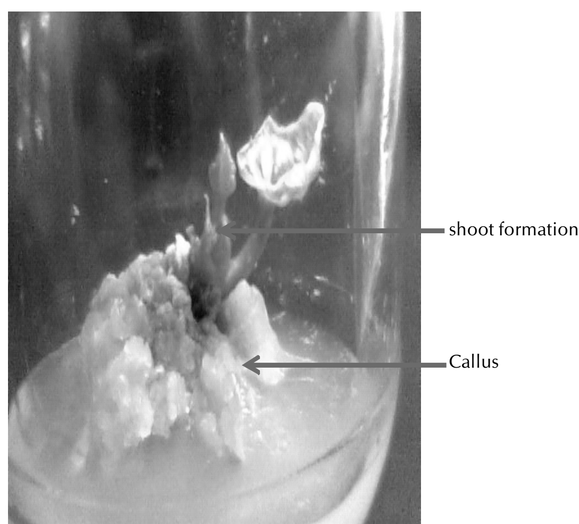

Figure 2: Callus differentiation in MS medium supplemented with BAP (1.5 mg/L) and IBA (1.0 mg/L)

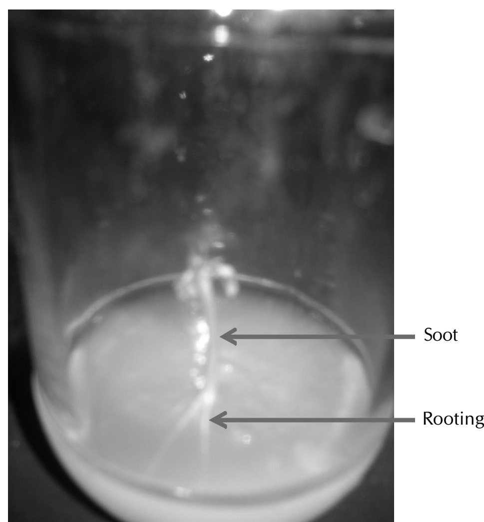

Figure 3: Root induction in MS medium supplemented with IBA (3.0 mg/L)

(1.5 mg/L) and IBA (1.0 mg/L) was best for callus induction (Fig. 2). The callus so produced was compact and green. It grew profusely and possessed high regeneration potential.

Rooting and plantlet establishment

In vitro shoots when transferred to ½ MS strength medium with IBA (2.0-3.0 mg/L) and NAA (1.0-4.0 mg/L), the rooting was comparatively better in ½ strength MS medium with IBA. Root initiation started after two weeks of culture on rooting medium with (3.0 mg/L) IBA and showed best rooting with 3 fold increase in root length as compared to control (Fig. 6). While with ½ MS medium with NAA (1.0-4.0 mg/L) profuse callusing formation took place and no root formation occurred (Table 3).

Acclimatization

After 2-3 weeks of culture of shoots on rooting medium the plantlets were transferred to pots containing a mixture of garden soil mixed with vermiculite and sand (1:1:1) for hardening and acclimatization.

DISCUSSION

The objectives of present investigation were to develop an efficient protocol for shoot regeneration of *Jatropha curcas* and analyze the effect of hormonal combinations on callus differentiation. Embryo culture is one of the most widely used *in vitro* methods for shoot regeneration which involves the exploitation of embryo explants on basal media as used by Jyoti *et al*. (2000) and Jesus *et al*. (2003).

For callus differentiation the sterilized explants were transferred into solid MS media containing BAP, NAA, and IBA in various combinations and concentrations. Rajore and Batra (2005) used various auxins viz. IAA, IBA, NAA which were supplemented separately at different concentrations (0.5-5.0

Table 3: Effect of various growth regulators on rooting and plantlet establishment

Media½ MS	Hormonal combination (mg/L) NAA	IBA	Time taken in Weeks	Length of root cm.)	No. of roots	Survival rates
Control(M1)	-	-	3-4	0-0.5	0-1	0%
M2	1.0	-	2-3	1-1.5	2-3	60%
M3	3.0	-	1-2	2-2.5	3-4	80%
M4	4.0	-	2-3	1-1.5	1-3	75%
M5	2.0	2.0	1-2	1.5-2	3-4	90%
M6	-	3.0	1-2	2-3	4-5	95%

S. Ed. (±) 4.417; CD at 5% 8.926

mg/L) to the MS basal medium. NAA was found to be the best for producing green, compact and fast growing callus, the optimal concentration being (1.0 mg/L). Similar result was reported by Sujatha and Mukta (1993). Present work has some variations with their result showing combination of BAP and IBA to be best for callus induction in media containing BAP (1.5 mg/L) and IBA (1.0 mg/L) after 25 days in light condition. Increasing or decreasing this concentration affected the callusing negatively. This result is in accordance with Thepsamran *et al.* (2006).

For shoot regeneration the sterilized callus were cultured on solid MS media containing BAP, IBA and NAA in various combinations and concentrations. The best shoot induction was observed in cultures containing BAP (3.0 mg/L) and IBA (1.0 mg/L) in light condition after 15-20 days of inoculation of callus. Increasing and decreasing this concentration reduced the no. of shoot buds. The cultures kept in dark condition showed very insignificant response. Shoot induction was also observed in the cultures containing NAA (0.5 mg/L) and BAP (2.0 mg/L) kept in light condition after 20-25 days of inoculation. Similar results were obtained by Srivastava and Banerjee (2008). Some other workers also have reported a combination of cytokinin and auxin to be fruitful for clonal propagation as by Joshi and Dhar (2003).

In vitro shoots when transferred to ½ MS strength medium with IBA (2.0-3.0 mg/L) and NAA (1.0-4.0 mg/L), the rooting was comparatively better in ½ strength MS medium with IBA. Root initiation started after two weeks of culture on rooting medium with (3.0 mg/L) IBA which showed best rooting with 3 fold increase in root length as compared to control. While with ½ MS medium with NAA (1.0-4.0 mg/L) profuse callusing took place and no root formation occurred. The present result has confirmed the findings of Scott (1972).

ACKNOWLEDGEMENT

The authors are highly thankful to the Department of Tissue Engineering, JSBB, SHIATS for providing the necessary laboratory and library facilities.

REFERENCES

Dastur, J. F. 1952. *Medicinal plants of India and Pakistan, Jatropha curcas,* 159, D. B. Taraporevela Sons and Co. Ltd., Bombay.

Datta, S. K. and Pandey, R. K. 1993. *Applied Botany Jatropha curcas. Abstracts.* **1**: 108-118.

Jesus, A. M. S., Pasqual, M., Dutra, L. F. and Chagas, E. A. 2003. *In vitro* culture of *Jatropha curcas* zygotic embryos. *Revista-ceres.* **50:** 183-189.

Johri, B. M. and Srivastava, P. S. 1973. Morphogenesis in endosperm cultures. *Z. Pflanzenphysiol.* **70:** 285–304.

Joshi, M. and Dhar, U. 2003. *In vitro* propagation of Saussurea obvallata (DC) Edgew. An enthnoreligious medicinal herb of Himalaya. *Plant Cell Rep.* **21:** 933-939.

Jyoti, S., Batra, A. and DJ, A. 2000. An expenditions method for regeneration of somatic embryo in *Jatropha curcas* L. *Phytomorphology* **59:** 242-289.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15:** 473–479.

Pierik, R. L. M. 1991. Commercial aspects of micropropagation, In Horticulture – *New Technologies and Applications* (Eds Prakash, J. K. and Pierik, R. L. M.), Dordercht, The Netherlands, "Micropropagation- Definitions from Dictionary. com". dictionary. reference. com. Retrieved 2008. pp. 03-17.

Qin, W., Wei-Da, L., Yi, L., Shu-Lin, P., Ying, X. U., Lin, T. and Fang, C. 2004. Plant regeneration from epicotyl explants of *Jatropha curcas. J. Plant Physiol. Mol. Biol.* **30:** 475–478.

Rajore, S. and Batra, A. 2005. Efficient plant regeneration via shoot tip explant in *jatropha curcas* L. *J. Plant Biochemistry and Biotechnology.* **14: 73-75.**

Srivastava, P. S. 1971. *In vitro* induction of triploid roots and shoots from mature endosperm of *Jatropha panduraefolia*. *Z. Pflanzenphysiol.* **66:** 93–96.

Srivastava, P. S. and Johri, B. M. 1974. Morphogenesis in mature endosperm cultures of *Jatropha panduraefolia*. *Beitr. Biol. Planz.* **50:** 255–268.

Srivastava, S. and Banerjee, M. 2008. In vitro clonal propagation of physic nut (*Jatropha curcas* L.) Influence of additives. *International J. Integrative Biology.* **3:** 73-79.

Sujatha, M. and Dhingra, M. 1993. Rapid plant regeneration from various explants of *Jatropha integerrima*. *Plant Cell Tissue Organ Cult.* **35:** 293–296.

Sujatha, M. and Mukta, D. 1993. Rapid plant regeneration from various explants of *Jatropha itegerrima*. *Pl. Cell Tiss. Organ cult.* **35:** 293-296.

Sujatha, M. and Mukta, N. 1996. Morphogenesis and plant regeneration from tissue cultures of *Jatropha curcas. Plant Cell Tissue Organ Cult.* **44:** 135–141.

Sujatha, M., Makkar, H. P. S. and Becker, K. 2006. Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha* curcas L. *Plant Growth Reg.* **47:** 83–90.

Thepsamran, N., Thepsithar, C. and Thongpukdee, A. 2006. *In vitro* multiple shoot induction of physic nut (*Jatropha curcas*) Nakhon Pathom: Department of Biology, Faculty of Science, Silpakorn University, Thailand.

Yoshifumi, T. 1982. Development study on Jatropha curcas oil as a substitute for diesel engine oil in Thailand. *Interim Report. Agric. Engineering Div. Department of Agriculture, Thailand.*